PROCESS FOR ANALYZING LENGTH POLYMORPHISMS IN DNA REGIONS

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

This application is *a reissue application of U.S. Pat. No. 5,766,847, issued Jun. 16, 1998 from application Ser. No. 08/145,617, filed Nov. 4, 1993, which is* a continuation of [application Ser.] *application* No. 07/681,494 filed on Jun. 10, 1991, now abandoned. *Application Ser. No. 07/681,494 is the national stage under 35 U.S.C. §371 of International Application PCT/EP89/01203, filed Oct. 11, 1989 and designating the United States.*

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to a process for determining identity and kinship of organisms on the basis of length polymorphisms in the regions of simple or cryptically simple DNA sequences.

2. Description of Related Art

All usual processes for the determination of identity and kinship on the basis of DNA length polymorphisms are based on the use of restriction endonucleases. Thereby specific DNA fragments are prepared which are afterwards detected by means of hybridization methods. With these methods either variations in length which have formed between the corresponding recognition sites for restriction endonucleases or variations in length which, have formed due to the lack of certain restriction cleavage sites are analyzed. The first type of polymorphism analysis reveals the variation in length in so-called minisatellite regions (**3**, **4**, **4**a, **4**b) and/or in regions with specific simple DNA sequences (**5**). The second analysis in which restriction fragment length polymorphisms (RFLP) due to the presence or absence of a restriction site, are detected can be applied only in specific, empirically found cases and can substantially be used appropriately only in the analysis of genetic diseases.

The disadvantage of both known methods lies in the fact that a hybridization reaction has to be carried out to make the length polymorphic regions visible. This makes the methods time-consuming and expensive. Furthermore, a single analysis using the previous methods does normally not allow any definitive conclusion about the relationship of two samples to be made so that additionally a second independent analysis becomes necessary. Therefore, these processes are not very appropriate for serial examinations and routine testing. Furthermore, the described method are not suitable for automation.

Higuchi et al. (**5**a) describe a further process for analyzing a length polymorphic locus, comprising a primer-controlled polymerization reaction of certain mitochondrial DNA sequences. This process cannot be used for paternity determination due to the mitochondrial markers used thereby.

Thus, it is the object of the present invention to provide a method for analyzing length polymorphisms in DNA regions which is highly sensitive, achieves reliable results without being time-consuming, is furthermore appropriate for serial examinations and routine testing and can optionally also be carried out automatically.

According to the invention this problem is solved by providing a process for determining identity and kinship of

organisms on the basis of length polymorphisms in DNA regions, which process comprises the following steps:

(a) annealing at least one primer pair to the DNA to be analyzed, wherein one of the molecules of the primer pair is substantially complementary to one of the complementary strands of the DNA flanking a simple or cryptically simple DNA sequence on either the 5' or the 3' side, and wherein the annealing occurs in such an orientation that the synthesis products obtained by a primer-directed polymerization reaction with one of said primers can serve as template for annealing the other primer after denaturation;

(b) primer-directed polymerase chain reaction; and

(c) separating and analyzing the polymerase chain reaction products.

In this process the individual primer molecules of the primer pairs are annealed to the DNA region to be analyzed at a distance of 50 to 500 nucleotides apart so that they encompass it at the given distance. Thereby the DNA region to be analyzed is surrounded by the hybridization molecules of the primer pair.

The primer-directed chain reaction is known as such from EP-A2 0 200 362 (**1**), from EP-A1 0 237 362 (**1**a) and from (**2**). It refers to a process for amplification of specific DNA fragments in which a PCR (polymerase chain reaction) is carried out. In this process the specific amplification is achieved by using oligonucleotide primers flanking the target-molecule in an anti-parallel manner. Thereby in a template-dependent extension of the primers by a polymerase DNA fragments are synthesized which themselves are again available as templates for a new cycle of primer extension. The DNA synthesis is performed by heat denaturation of the starting molecules, followed by hybridization of the corresponding primers and by chain extension with a polymerase. By means of a further heat denaturation a following cycle is then performed. Thereby the specifically amplified region grows in an exponential way and finally a fragment detectable by normal gel electrophoresis is formed. The length of this fragment is determined by the length of the primers and the intermediate region and is similar or equal to the sum of the lengths of the primers and the intermediate region. The use of thermostable synthesis components allows control of the process by simple and easily automated heating and cooling cycles.

By "antiparallel flanking" of the target molecule by oligonucleotide primers one understands the hybridizing of one of both primers of a primer pair each to the complementary strands of the target molecule so that the 3' ends of the primer pair point at each other.

In (**15**) Marx describes different applications of the PCR process.

Rollo et al. describe in (**16**) the use of the PCR process for distinguishing between various species of the plant pathogenic fungus Phoma.

The use of simple and cryptically simple DNA sequences in the fragment of PCR processes for determining identity and kinship of organisms is not described in any of these references.

SUMMARY OF THE INVENTION

Simple and cryptically simple DNA sequences are repetitive components of all eukaryotic genomes which to some extent can be found also in prokaryotic genomes (**6**–**9**). Thereby simple DNA sequences comprise short DNA motifs containing at least one nucleotide and not more than approximately 6 to 10 nucleotides arranged as a dozen to